BIOLOGICAL NITROGEN FIXATION

Large-Scale Assessment of Symbiotic Dinitrogen Fixation by Crops: Soybean and Alfalfa in the Mississippi River Basin

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ABSTRACT

Human activities have increased the amount of earth's reactive N, resulting in significant improvements in crop yield and animal production but also in environmental degradation and ecosystem disruption in some areas. For example, agriculture has been cited as a major source of N that contributes to hypoxia in the Gulf of Mexico. Although other sources of N have been well characterized in large ecosystem studies, the contribution of legume crops to the N cycle has not. Furthermore, the role legumes play in reducing excess N is not widely recognized. Symbiotic N_2 fixation is a facultative process that is reduced by plant N uptake from other sources. Using reported and estimated crop yield and protein concentration with published estimates of soil N mineralization and atmospheric N deposition, we estimated spatial patterns of symbiotic N_2 fixation for soybean [*Glycine max* (L.) Merr.] and alfalfa (*Medicago sativa* L.) across the Mississippi River Basin, the largest in North America. We estimate that alfalfa haylage adds about 20% to total production of dry alfalfa hay and increases total land area in alfalfa by about 11% over that reported in the Census of Agriculture. Our analysis shows wide ranges in N_2 fixation (0 to 185 kg N ha^{-1} for soybean and 45 to 470 kg N ha^{-1} for alfalfa), reasonable mean rates (84 kg N ha^{-1} for soybean and 152 kg N ha^{-1} for alfalfa), and suggests that about 2.9 million Mg of fixed N is harvested annually in these two cultivated legumes.

Biological N_2 fixation by leguminous plants is a significant source of available N in both natural and managed ecosystems (Galloway et al., 1995). Annual rates of symbiotic N_2 fixation by cultivated legumes are often at least one order of magnitude higher than rates of N_2 fixation in natural ecosystems (Cleveland et al., 1999; Ledgard and Giller, 1995). Cultivated legumes reportedly still provide nearly as much N annually to agricultural systems as N fertilizer although global inputs by fertilizer have recently exceeded those of N_2 fixation (Vitousek et al., 1997).

Traditionally, legumes have been viewed as excellent sources of N in agriculture (Kinzig and Socolow, 1994). Dinitrogen fixation by legumes is a facultative process—less N_2 is fixed by the rhizobia as N supply from other sources increases (Allos and Bartholemew, 1959; Cherney and Duxbury, 1994; Blumenthal et al., 1999). Important sources of inorganic N include mineralized soil organic matter, current or residual fertilizer and manure N, mineralized crop residue N, and dry and wet deposition of atmospheric N.

A major limitation of ecosystem-scale estimates of N_2 fixation is that they usually are based on mean fixation rates determined in research trials (e.g., Galloway et al., 1995; Vitousek et al., 1997; Mitsch et al., 2001; Boyer et al., 2002; McIsaac et al., 2002). In fact, symbiotic N_2 fixation varies considerably among years (Heichel and Henjum, 1991) and within fields (Walley et al., 2001), due to biological and physical constraints that affect yield potential (N demand) and soil N availability (N supply). Cleveland et al. (1999) estimated N_2 fixation in natural ecosystems based on evapotranspiration, a surrogate for aboveground dry matter accumulation, but we found no published estimates of large-scale spatial patterns of N_2 fixation rates by important agricultural legumes.

In addition to helping define N input in ecosystem studies, spatial patterns of N_2 fixation could be used to understand the potential for cultivated legumes to reduce excess ecosystem N (Johnes and Butterfield, 2002). Our objective was to estimate symbiotic N_2 fixation by soybean and alfalfa, the most important grain and forage legume crops in the Mississippi River Basin, the largest river basin in North America and the third largest on earth.

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MATERIALS AND METHODS

We estimated symbiotic N_2 fixation as the difference between harvested N and the uptake of inorganic N in each crop. Reported crop yield by county or parish and reported or estimated N (or protein) concentration in harvested beans or forage provided the basis for calculating harvested N. Nitrogen supply from sources other than N_2 were limited to soil organic matter mineralization and terrestrial deposition of atmospheric N (background wet deposition amounts measured in the National Atmospheric Deposition Program/National Trends Network plus estimated redeposition of locally derived ammonia from manure, fertilizers, and plant senescence). We used estimates of N supply from soil organic matter mineralization and atmospheric N deposition that were derived by Burkart and James (1999). We assumed a net 80% efficiency in plant uptake and assimilation into harvested tissues from these sources and assumed that the remainder was utilized in nonharvested plant residues, immobilized by soil microorganisms, and lost by various pathways. We calculated crop N harvested for each hydrologic unit in the Basin assuming proportional representation by the area of each county present in the hydrologic unit. Hydrologic units are "river basins having drainage areas usually greater than 700 square miles [1800 km^2]" (USDA-NRCS, 1998).

Soybean Yield and Nitrogen

Soybean yield was derived for each county in the Mississippi River Basin from Census of Agriculture data for 1997 (USDA-

Abbreviations: NASS, National Agricultural Statistics Service.

NASS, 1999). In a few cases where disclosure rules resulted in missing data for harvested area or production, we used available data from the 1992 census. No soybean data were reported for Idaho, Colorado, Montana, New Mexico, and Wyoming. The Soybean Quality Survey of the American Soybean Association and the United Soybean Board provides measurements of protein concentrations in beans from most states (Iowa Grain Quality Initiative, 2002). We used the mean protein concentration for 1997 through 2002 to obtain larger sample sizes. Mean statewide soybean protein concentration ranged from 336 g kg^{-1} in North Dakota to 375 g kg^{-1} in Georgia. The mean of concentrations reported for Ohio and Virginia was used for West Virginia. Total N concentration in beans was calculated by dividing protein concentration by 6.25. Nitrogen yield in beans was calculated as the product of dry mass yield and N concentration.

Alfalfa Yield and Nitrogen

Unlike the case with soybean, where reported bean yield accounts for nearly all the harvested crop, alfalfa is harvested as several products: hay, haylage (silage), green chop (cut and fed directly to livestock), and grazed pasture. The latter two are considered minor in most states. Alfalfa hay was harvested from about 5.7 million ha in the Mississippi River Basin in 1997 (USDA-NASS, 1999). All states in the Basin report alfalfa hay production, except Mississippi, where little alfalfa is grown due to insect and disease problems (J. Larry Oldham, personal communication, 2003). In a few cases where disclosure rules resulted in missing data for alfalfa area or production, we used available data from the 1992 census. Where only alfalfa area or production was reported for a county in 1997, we used the yield of surrounding counties to estimate yield and then calculated the other missing component (production or area) for the county in question.

Alfalfa haylage has become increasingly important nationwide although the National Agricultural Statistics Service (NASS) reports state-level alfalfa haylage data for only six states in the Mississippi River Basin (Michigan, Minnesota, New York, Pennsylvania, West Virginia, and Wisconsin). Only for Wisconsin is alfalfa production expressed as the total of hay and haylage production and is reported at the county level. To help fill this gap in the data, Extension forage specialists in several states provided estimates of statewide haylage production. Haylage production figures were estimated for remaining states by extrapolating from these and NASS statistics. We estimated haylage-only land area in states east of about 96° W longitude based on the following relationship derived from state-level NASS data:

$$y = 0.428x + 0.00505x^2 \ (R^2 = 0.94)$$

where y = alfalfa haylage-only land area/total alfalfa area and x = alfalfa haylage production/total alfalfa production. In all cases where only state-level estimates were available, we apportioned these to the various counties or parishes by the proportional distribution of dairy cows. Where county or parish data for dairy cow number were not available, we used alfalfa hay numbers alone. Where alfalfa hay data were unavailable for a county or parish, no alfalfa haylage production was predicted.

The amount of harvested N in alfalfa forage was calculated from data provided by public and private laboratories and from estimates provided by Extension specialists of crude protein concentration in alfalfa hay and haylage. The range in reported crude protein concentration was 187 to 220 g kg^{-1} on a dry mass basis (Table 1). Alfalfa N concentration was calculated by dividing mean statewide crude protein concentration by 6.25. Nitrogen yield was taken as the product of N concentration and alfalfa dry matter production.

RESULTS AND DISCUSSION

Estimated Haylage Production

An important outcome of our research is the estimation of alfalfa haylage production. Haylage production

Table 1. Alfalfa hay and haylage production in 1997 by state for counties comprising the Mississippi River Basin, and estimated average crude protein concentration. List includes only states with at least 10 000 ha of alfalfa in the Basin. Data for hay were published by the National Agricultural Statistics Service (NASS); haylage figures were derived from the indicated sources. Crude protein concentration was derived from laboratory databases and estimates from Extension specialists.

State	Land area: Hay	Land area: Haylage only	Source†	Production: Hay	Production: Haylage	Production: Haylage: total	Crude protein
	1000 ha			1000 Mg hay equivalent		%	g kg^{-1}
Colorado	227	0	Ext.	1780	94	5	213
Illinois	182	48	Ext.	1360	732	35	200
Indiana	122	25	Extrap.	843	361	30	187
Iowa	438	38	Ext.	3097	546	15	199
Kansas	322	0	Extrap.	2839	149	5	208
Kentucky	93	11	Ext.	554	139	20	210
Minnesota	432	82	NASS	2822	891	24	200
Missouri	152	10	Extrap.	930	127	12	196
Montana	597	0	Ext.	2826	58	2	200
Nebraska	489	10	Extrap.	3418	180	5	207
New Mexico	21	0	Ext.	135	7	5	220
New York	13	9	NASS	73	93	56	201
North Dakota	451	9	Extrap.	1642	86	5	196
Ohio	193	64	Ext.	1224	816	40	205
Oklahoma	122	0	Extrap.	967	51	5	216
Pennsylvania	109	31	NASS	613	444	42	201
South Dakota	838	17	Extrap.	4054	213	5	200
Tennessee	16	1	Ext.	101	14	12	210
Texas	20	0	Extrap.	164	3	2	214
Virginia	16	1	Extrap.	101	18	15	201
West Virginia	18	2	NASS	97	16	14	201
Wisconsin	599	315	NASS	3913	3685	49	200
Wyoming	233	0	Ext.	1240	25	2	201

† Ext., estimates of haylage production and/or area from Extension specialists in the area; NASS, published estimates from USDA-NASS; Extrap., estimate based on extrapolation from neighboring states.

was below 10% of total alfalfa in the western portion of the Mississippi River Basin (states west of about 96° W longitude) but ranged up to 56% in the eastern portion (Table 1). Our analysis shows that total alfalfa production in the Mississippi River Basin likely exceeds reported alfalfa dry hay production by 20% and that land area devoted to alfalfa production totals about 6.4 million ha, about 11% larger than reported for alfalfa in the Census of Agriculture for 1997. In our view, it would be beneficial for more states in humid areas to collect statistical data on alfalfa haylage production.

Estimated Symbiotic Dinitrogen Fixation

About 1.24 million Mg of total N was harvested in hay and haylage from alfalfa fields in the Mississippi River Basin in 1997, an average of 193 kg N ha^{-1} (Fig. 1). Of this, we estimate that an average of 79% (152 kg N ha^{-1}) was derived from symbiotic N_2 fixation. These figures vary across the 787 watersheds where alfalfa was grown in the Basin, with N_2 fixation ranging from 43 to 471 kg N ha^{-1}. We estimate that between 29 and 99% of crop N was derived from N_2 fixation. Although no data were available (due to NASS restrictions on publication) for areas of northern Texas where alfalfa likely has very high N_2 fixation rates, our estimated mean regional N_2 fixation rate falls within the 130 to 335 kg N ha^{-1} used by others (Peterson and Russelle, 1991; Boyer et al., 2002; McIsaac et al., 2002), and the range is consistent with site-specific estimates in the literature of 45 to 450 kg N ha^{-1} (Russelle, 2005). Ours is the first report for alfalfa that emphasizes the spatial variability of N_2 fixation across a large region.

Soybean is the most widely grown grain legume in the Mississippi River Basin, occupying about 23 million ha (USDA-NASS, 1999). It is grown in rotation with other annual crops, such as corn (*Zea mays* L.), wheat (*Triticum aestivum* L.), or potato (*Solanum tuberosum* L.). Soybean production has increased 10-fold in the USA since 1950 (USDA-NASS, 2003a). Thus, its effect on N cycling in the Basin has increased substantially over the past three decades. Total soybean production yielded about 3.4 million Mg of N in the Basin in 1997, averaging 147 kg N ha^{-1} (Fig. 2). We estimate that

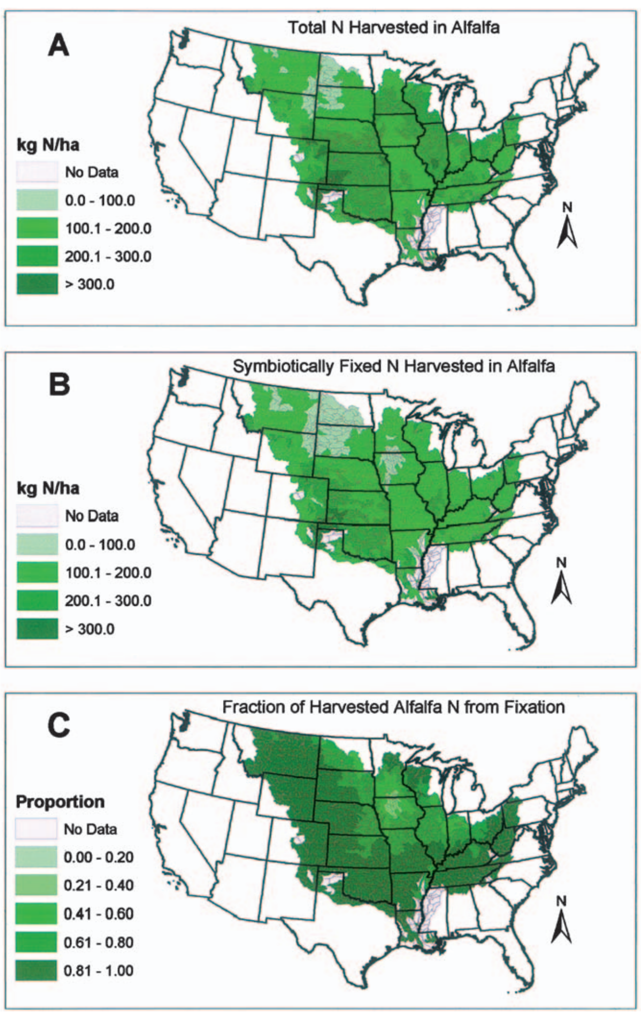

Fig. 1. Spatial patterns of (A) total N harvested, (B) rate of symbiotic N_2 fixation, and (C) fraction of total harvested N derived from symbiotic N_2 fixation in alfalfa hay and haylage in the Mississippi River Basin in 1997. Rates are expressed per hectare of harvested alfalfa and plotted for hydrologic units.

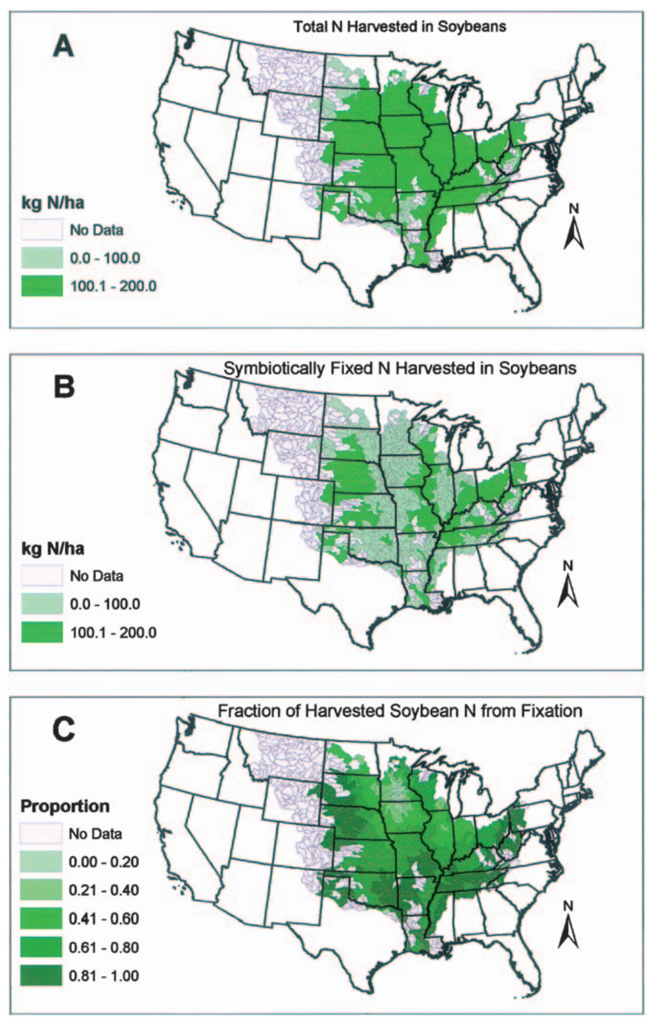

Fig. 2. Spatial patterns of (A) total N harvested, (B) rate of symbiotic N_2 fixation, and (C) fraction of total bean N derived from symbiotic N_2 fixation in soybean in the Mississippi River Basin in 1997. Rates are expressed per hectare of harvested soybean and plotted for hydrologic units.

an average of 57% (84 kg N ha^{-1}) was derived from symbiotic N_2 fixation. There was high variation in estimated N_2 fixation rate across the 570 watersheds in the Basin where soybean was grown, ranging from 0 to 185 kg N ha^{-1}. The fraction of bean N derived from symbiotic N_2 fixation ranged from 0 to 96%. These estimates fall well within regional mean rates used by others (Boyer et al., 2002; McIsaac et al., 2002) and correspond to site-specific reports in the literature, which range from 13 to 188 kg N ha^{-1} (Russelle, 2005), but our results demonstrate spatial relationships not previously available for soybean.

The estimated amount of symbiotically fixed N totaled about 1.9 million Mg in soybean and about 1.0 million Mg in alfalfa forage in the Mississippi River Basin. At the same time, a total of about 1.7 million Mg of N from soil and atmospheric deposition was harvested in these crops.

Constraints to the Dinitrogen Fixation Estimates

Although there are numerous uncertainties in our estimates of N pools that contribute to soybean and alfalfa N uptake, many have opposite effects on estimated N_2 fixation and likely have only a marginal effect on our estimates.

Our estimates of symbiotic N_2 fixation are based on only the latest year of county-level data available from the Census of Agriculture. On the scale of fields and hydrologic units, yields will vary from year to year due primarily to weather and crop pests, and the area planted will vary with market prices, government programs, and other factors. No single year will therefore be "typical" for the entire Basin. Plots of state-level historical crop area, yield, and production (USDA-NASS, 2003b) showed that, with some exceptions, results for alfalfa in the 5 yr before and after 1997 fell within 33% of the 1997 values (Fig. 3). The largest consistent deviation for yield (directly related to our estimate of N_2 fixation) was an underrepresentation for alfalfa in North Dakota in 1997 compared with other years. Larger differences were apparent for soybean than alfalfa. The area planted to soybean increased markedly in most years since 1997 in Nebraska, South Dakota, and Wisconsin but declined to about 50% of 1997 area in Louisiana. Production was more than 33% higher than 1997 in four of the five succeeding years in Wisconsin and was 33 to about 50% lower in Louisiana, Mississippi, and Oklahoma. With these exceptions, the estimates of N_2 fixation we present here appear to be reasonably accurate for most states in the Basin.

Our estimates rely on the general validity of estimated soil N mineralization and ammonia redeposition from local sources (Burkart and James, 1999). Soil N mineralization will vary from year to year but is likely to be higher under conditions that also increase crop yield, reducing the effect on our estimates of N_2 fixation. Patterns of wet and dry ammonia redeposition in agricultural areas are not known with certainty. They are likely to change primarily as concentrated animal feeding operations come on line or are abandoned but also will be affected by use of different fertilizer N sources like anhydrous ammonia and urea. Burkart and James (1999) relied on relationships developed by Ferm (1998) for ammonia redeposition under European conditions, which may not apply in the central USA. Although their estimates have been questioned (McIsaac et al., 2002), a large area of the region may be receiving between 23 and 40 kg N ha^{-1} from ammonia redeposition (Burkart and James, 1999), and it would be advisable to establish monitoring networks to verify these estimates.

An important uncertainty in our estimates is the assumption of inorganic N uptake by the legumes. We selected 80% recovery from mineralized soil N and deposition of atmospheric N because both crops efficiently absorb inorganic N present in the soil (Heichel and Barnes, 1984; Herridge et al., 1990; Varvel and Peterson, 1992; Daliparthy et al., 1995; Randall et al., 1997; Schmidt et al., 2000; Goss et al., 2002) and both efficiently absorb atmospheric ammonia (Harper et al., 1989; Dabney and Bouldin, 1990). For example, little nitrate loss from subsurface tile drains occurs under alfalfa, regardless of water flux (Randall et al., 1997). It is reasonable to assume that alfalfa absorbs most of the slowly released mineralized soil N although some mineralized N may be immobilized by rhizosphere organisms that are utilizing root exudates. In contrast, Cambardella et al. (1999) concluded that significant nitrate losses in tile drainage occur in both phases of the corn–soybean rotation because of "asynchronous production and uptake of NO_3–N in the soil and the presence of large quantities of potentially mineralizable N in the soil organic matter" (p. 25) in soils of the Des Moines lobe glacial till that they studied. Thus, our estimates of inorganic N uptake by soybean may be too high for areas where significant nitrate leaching occurs when soybean is not actively growing, such as on tile-drained or coarse-textured soils. Although we are less confident in our assumption of 80% recovery of available N by soybean than by alfalfa, it resulted in reasonable estimates of N_2 fixation when inorganic N supply was high, i.e., minima near 0 kg N ha^{-1} for soybean and 43 kg N ha^{-1} for alfalfa. Field data from experiments that explicitly measure efficiency of N uptake from mineralized soil N and atmospheric deposition are needed to test our assumption.

Our estimates may also be affected by the uncertainty in amounts of residual N available from fertilizer and manure applied to previous crops. This is a particular problem with soybean because it is an annual crop that typically is grown in rotation with nonlegumes that receive fertilizer and/or manure N. There are no site-specific data on residual inorganic N for the Mississippi River Basin. Residual inorganic N is less problematic with a perennial crop like alfalfa, which usually is grown for three or more years (Peterson and Russelle, 1991).

Similarly, our estimate of N_2 fixation removed in alfalfa and soybean may be somewhat high because it does not include livestock manure already being applied to these crops and ignores nitrate available in subsoil, shallow groundwater, or irrigation water to deeply rooted alfalfa. No quantitative estimates of these sources were found for the region. Alfalfa efficiently absorbs and

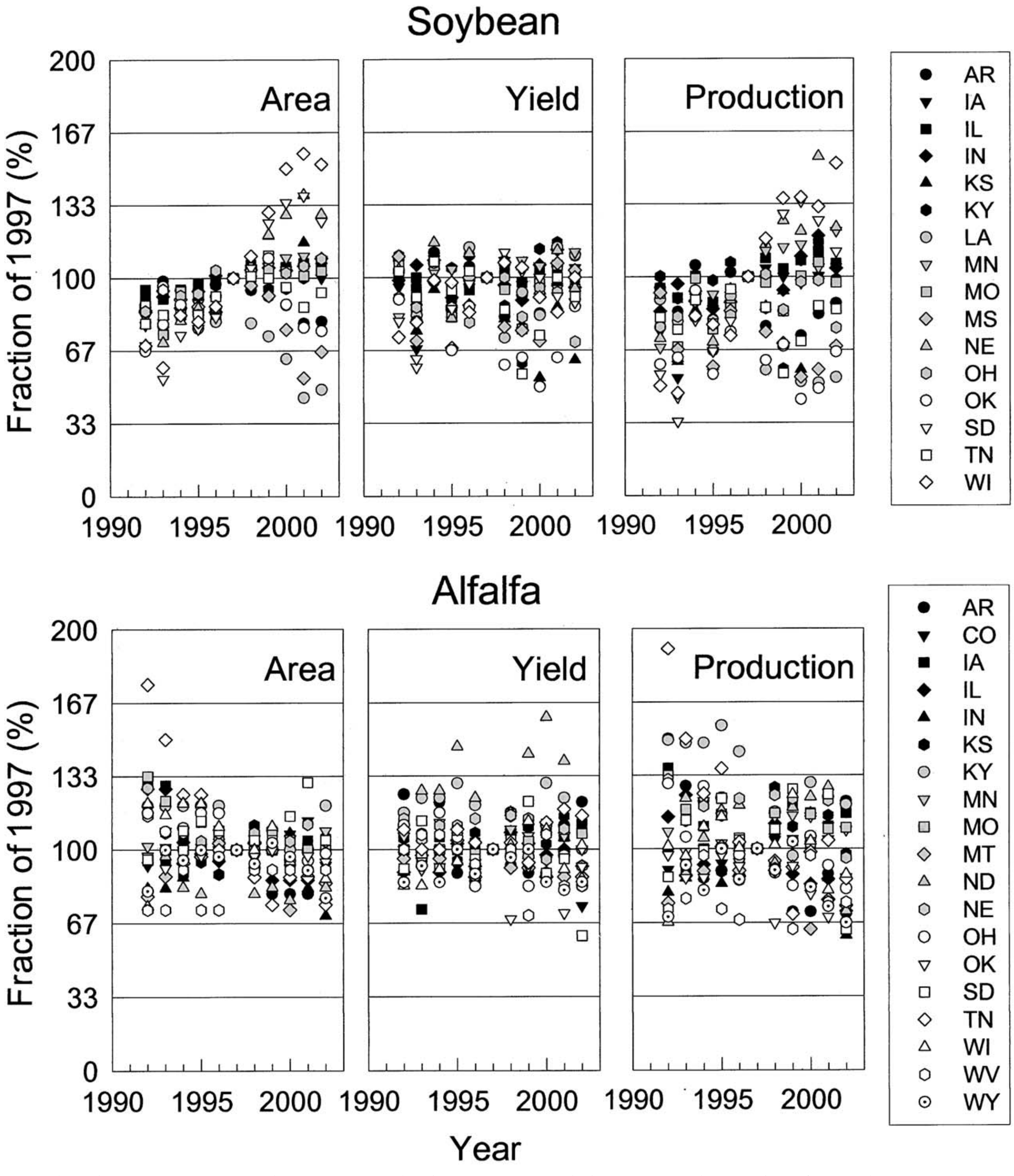

Fig. 3. Fraction of harvested crop area, yield, and production for soybean and alfalfa compared with data from 1997, which were used to estimate symbiotic N_2 fixation. Data were obtained from USDA-NASS (2003b) for statewide totals (area and production) and means (yield). Note that the legend differs between crops because data from different states were included.

utilizes these N sources and reduces the amount of N fixed by similar amounts (Russelle et al., 2001; Russelle et al., unpublished data, 2003). Although one-third to one-half of dairy farmers in Minnesota and Pennsylvania reported that they apply some manure to perennial forages, like alfalfa (Russelle, 1997; Dou et al., 2001), we do not know whether farmers in other Mississippi River Basin states follow similar practices. In the Minnesota case (Russelle, 1997), many of these reports appeared to reflect manure applications to alfalfa shortly before rotating to another crop, in which case N_2 fixation by the alfalfa would not be altered. Apparently, few producers apply manure to soybean (Schmitt et al., 1999).

On the other hand, our estimate of N_2 fixation may be low because it does not include fixed N in the root system (Dubach and Russelle, 1994; Ta and Faris, 1987), fixed N in alfalfa or soybean shoot residues that remain in the field, and removal of alfalfa by grazing. The constraint of ignoring nonharvested plant parts may not affect estimates of N_2 fixation by soybean because relatively little N remains in soybean plant residues after harvest (Bergersen et al., 1989; Ravuri and Hume, 1993). In alfalfa, one-third to one-half of the fixed N in roots and crowns is remobilized to the shoots during early regrowth after herbage harvest (Ta and Faris, 1987; Russelle et al., 1994; Ourry et al., 1994), reducing the size of this belowground pool of fixed N, but this is counterbalanced by an estimated annual transfer to the soil of 20 to 60 kg ha^{-1} of N from all sources by fine-diameter root turnover (Goins and Russelle, 1996). In pure alfalfa stands, recycled N from root turnover and surface residue decomposition is reabsorbed by alfalfa, added to the soil organic N pool, or lost by various pathways.

The approach we used for estimating symbiotic N_2 fixation by cultivated legumes is rather simple and could be applied to other areas and legumes. Although there are uncertainties associated with this method, we believe it provides reasonable large-scale estimates that illuminate spatial relationships. It may be more difficult to use with mixed stands of legumes and nonlegumes, but modeling approaches could be employed in that case to estimate the partitioning of soil inorganic N uptake between the species (Schwinning and Parsons, 1996; Vitousek et al., 2002).

CONCLUSIONS

Symbiotic N_2 fixation by soybean and alfalfa varies spatially across the Mississippi River Basin. Our analysis is significant because it demonstrates that these differences are likely to be very large within the region, even if the absolute amounts we calculated are not correct. For example, although alfalfa yields are high in south-central Minnesota, N_2 fixation is relatively low in response to high inorganic N supply—we estimate that less than 20% of the harvested N in alfalfa is derived from N_2 fixation in that area. In contrast, where forage yield is high and inorganic N supply is low, N_2 fixation by alfalfa appears to be greater than 400 kg N ha^{-1}. Therefore, the use of Basin-wide means of N_2 fixation is not appropriate for analyses of N sources and cycling within subwatersheds.

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